

We claim:

1. A method for determining whether a bacteria is likely to be tolerant to at least one
5 antibiotic comprising

(a) determining whether said bacteria has a type 4 or R6 allele of the vex2 gene, and
(b) determining whether said bacteria has a type 4 or R6 allele of the pep27 gene,
wherein said bacteria is determined to be likely to be tolerant if it has a type 4 allele
of the vex2 gene and an R6 allele of the pep27.

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2. The method of claim 1 wherein said antibiotic is a β lactam.

15 3. The method of claim 1 wherein said antibiotic is selected from the group consisting of penicillin and vancomycin.

20 4. The method of claim 1 wherein the step (a) is accomplished by determining the nucleotides present in the vex2 gene of said bacteria at the locations corresponding to nucleotides 41 and 67 of SEQ ID No. 1, and step (b) is accomplished by determining the nucleotides present in the pep27 gene of said bacteria at the locations corresponding to nucleotides 35 and 46 of SEQ ID No. 3.

25 5. The method of claim 4 wherein steps (a) and (b) are accomplished by sequencing a region of the genomic DNA of said bacteria which includes said locations.

6. The method of claim 4 wherein steps (a) and (b) are accomplished by (i) amplifying a region of the genomic DNA of said bacteria which includes said locations to generate an amplified fragment, and (ii) treating the amplified fragment with a restriction enzyme in its corresponding restriction buffer to determine the identity of the nucleotide present at the selected locations.

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7. The method of claim 4 wherein steps (a) and (b) are accomplished by (i) amplifying a region of the genomic DNA of said bacteria which includes said locations, and (ii) hybridizing the amplified region with probes specific for the selected locations wherein hybridization determines the identity of the nucleotide present at the selected locations.

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8. The method of claim 1 further comprising determining whether said bacteria has a wildtype or tolerant allele of the vncS gene, wherein said bacteria is also determined to be tolerant if it has the tolerant allele of the vncS gene.

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9. A method for determining the vex2 genotype of a selected bacteria comprising determining the identity of the nucleotides located at the positions corresponding to nucleotides 41 and 67 of SEQ ID No. 1 in the genomic DNA of said selected bacteria.

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10. The method of claim 9 comprising the steps of:

- (a) isolating nucleic acid from the bacteria;
- (b) amplifying a region of the vex2 gene which includes the locations corresponding to nucleotides 41 and 67 of SEQ ID No. 1; and
- (c) determining the identity of the nucleotides located at the positions corresponding to nucleotides 41 and 67 of SEQ ID No. 1.

11. A method for determining the pep27 genotype of a selected bacteria comprising determining the identity of the nucleotides located at the positions corresponding to nucleotides 35 and 46 of SEQ ID No. 3 in the genomic DNA of said selected bacteria.

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12. The method of claim 11 comprising the steps of:

- (a) isolating nucleic acid from the bacteria;
- (b) amplifying a region of the pep27 gene which includes the locations corresponding to nucleotides 35 and 46 of SEQ ID No. 3; and

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(c) determining the identity of the nucleotides located at the positions corresponding to nucleotides 35 and 46 of SEQ ID No. 3.

13. A test kit suitable for determining the vex2 and pep27 genotype of a bacteria thereby
5 determining whether said bacteria is likely to be tolerant to at least one antibiotic comprising:

- (a) a predetermined amount of a first amplification primer complementary to a region of the vex2 gene 5' to the location corresponding to nucleotide 41 of SEQ ID No. 1;
- 10 (b) a predetermined amount of a second amplification primer complementary to a region of the vex2 gene 3' to the location corresponding to nucleotide 67 of SEQ ID No. 1; and
- (c) a predetermined amount of a third amplification primer complementary to a region of the pep27 gene 5' to the location corresponding to nucleotide 35 of SEQ ID No. 3; and
- (d) a predetermined amount of a fourth amplification primer complementary to a region of the pep27 gene 3' to the location corresponding to nucleotide 46 of SEQ ID No. 3;
- (e) other reagents; and
- (f) directions for use of said kit,

wherein said first amplification primer and said second amplification primer can be used to amplify a region of the vex 2 gene that distinguishes the type 4 and R6 alleles and said third amplification primer and said fourth amplification primer can be used to amplify a region of the pep27 gene that distinguishes the type 4 and R6 alleles.

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14. The kit of claim 13 wherein said first amplification primer is SEQ ID No. 7, said second amplification primer is SEQ ID No. 8, said third amplification primer is SEQ ID No. 9, and said fourth amplification primer is SEQ ID No. 10.

30 15. The kit of claim 13 further comprising:

(g) a predetermined amount of a fifth amplification primer complementary to a region of the vncS gene 5' to the location corresponding to nucleotide 79 of SEQ ID. No. 5; and

5 (h) a predetermined amount of a sixth amplification primer complementary to a region of the vncS gene 3' to the location corresponding to nucleotide 79 of SEQ ID. No. 5,

wherein said fifth amplification primer and said sixth amplification primer can be used to amplify a region of the vncs gene that distinguishes the wildtype and tolerant alleles.

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16. The kit of claim 15 wherein said fifth amplification primer is SEQ ID No. 11 and said sixth amplification primer is SEQ ID No. 12.

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